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CURRICULUM VITAE

Saswati Chatterjee

Division of Virology

City of Hope National Medical Center & Beckman Research Institute

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schatterjee@coh.org

EDUCATION

- | | |
|-------------|--|
| 1976 | B.Sc., McGill University, Montreal, CANADA |
| 1978 | M.Sc., McGill University, Montreal, CANADA |
| 1982 | Ph.D., McGill University, Montreal, CANADA |

RESEARCH EXPERIENCE

- | | |
|-----------------------|--|
| 1997-Present | Associate Research Scientist, Division of Pediatrics, City of Hope National Medical Center.
The use of AAV vectors for stem cell gene therapy. Biology of AAV vectors. Gene therapy of AIDS, cancer and cardiovascular diseases. |
| Dec 1991- 6/97 | Assistant Research Scientist, Division of Pediatrics, City of Hope National Medical Center and Dept. of Molecular Genetics, Beckman Research Institute of the City of Hope, Duarte CA. Development of AAV vectors for gene transfer into hematopoietic stem cells. Gene therapy of AIDS and Cancer. |
| 1990 - 1991 | Assistant Professor, Visiting Scientist, Division of Molecular Virology & Immunology, Dept. of Microbiology, Georgetown University School of Medicine, Rockville, MD. Transduction of intracellular resistance to virus replication (HIV-1 & HSV-1) using adeno-associated virus (AAV)-based vectors. |
| 1986 - 1990 | Visiting Associate with Dr. James Rose, Laboratory of Viral Diseases (Dr B. Moss, Lab Chief), NIAID, NIH, Bethesda, MD. Research in Molecular Virology: Induction of intracellular resistance to immunodeficiency viruses via transducing eukaryotic viral vectors. Development of an AAV-based vector system for gene transductions. DNA replication mechanisms of AAV. |
| 1985 - 1986 | Research Fellow with Dr. Michael J. Rogers, Laboratory of Genetics, NCI, NIH, Bethesda, MD. Molecular Biology of the wild mouse Major Histocompatibility Complex. |
| 1982 - 1985 | Visiting Fellow with Dr. David H. Sachs Transplantation Biology Section, Immunology Branch, NCI, National Institutes of Health, Bethesda, MD. Immunochemistry and Immunogenetics of the murine Major Histocompatibility Complex. |
| 1976 - 1982 | Graduate research with Dr. P.K. Lala, Department of Anatomy, McGill University. The Immunobiology of Feto-maternal relationships. |

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1975 - 1976 B.Sc. project: Biochemical Genetics, with Dr. P. Hechtman, Dept. of Biology, McGill University and MRC Human Genetics Group.

TEACHING EXPERIENCE

1994-Present City of Hope Graduate School faculty.

1980 - 1981 Gross Anatomy, Dept. of Anatomy, McGill University. Laboratory instructor to first year Medical students.

1980 - 1981 Surface Anatomy, Dept. of Anatomy, McGill University. Laboratory instructor to Physiotherapy Students

1979 - 1980 Medical Histology, McGill University. Laboratory instructor to first year Medical students.

1978 - 1979 Anatomy of the Head and Neck, Dept. of Anatomy, McGill University. Laboratory instructor to first year Dental students.

1972 - 1973 Mathematics - Teaching Assistant, Marianopolis College, Montreal.

HONORS & FELLOWSHIPS

1971 - 1973 Marianopolis College entrance and continuing scholarships

1973 - 1974 J.W. McConnell Scholarship in Science & Engineering, McGill University

1979 McGill University Faculty of Medicine Graduate Student Award

1979 - 1982 Conseil de la recherche scientifique du Quebec Graduate Studentship

1982 McGill University Dean's Honors' list

1982 Arthur W. Ham Graduate Student Award - Canadian Federation of Biological Societies.

1982 Mayo Foundation Fellowship.

1982 Medical Research Council Post Doctoral Fellowship.

1982 - 1985 Fogarty N.I.H. Visiting Fellowship.

1996 Moderator, oral presentation session on "Gene Therapy- Gene Transfer and Biology" American Society of Hematology 1996 annual meeting.

2001 Invited Faculty, American Society of Gene Therapy Annual meeting.

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Manuscript Reviews:

- Nature Medicine
- Blood
- Journal of Virology
- Human Gene Therapy
- Cancer Research
- Journal of Immunotherapy
- Pediatric Research
- Biotechniques

COMMITTEES

1993	Ad Hoc Reviewer, National Heart Lung Blood Institute, NIH. In vitro expansion of Hematopoietic Stem Cells.
1994	Ad Hoc Reviewer, National Institute of Allergy and Infectious Diseases, NIH. RFA: AI-93-12. Immune Reconstitution of HIV infected Individuals.
1996	Ad Hoc Reviewer, National Institute of Diabetes, Digestive and Kidney Diseases, NIH. RFA: DK: 95-006. Pathogenesis and Treatment of Cystic Fibrosis.
1996	Reviewer: American Society of Hematology 1996 annual meeting - Gene Therapy- Gene Transfer and Biology
1996-Present	City of Hope Research Animal Care Committee.
1998	Special Emphasis Panel: PAR-97-080 - Novel HIV therapies: Integrated preclinical/clinical program, NIAID, NIH.
1998	Special Emphasis Panel: PAR-98-007 Innovative Grant Program for Approaches in HIV Vaccine Research, NIAID, NIH.
1998	Special Review Panel: Gene Therapy Center Cores. NIDDK, NIH.
1999	Reviewer: American Society of Gene Therapy. Abstracts for presentation at 1999 annual meeting.
2000	Special Emphasis Panel: ZDK1 GRB-2 (M1). Correction of Hepatocytes with recombinant AAV for correction of genetic and metabolic abnormalities. NIDDK, NIH
2001	Reviewer: American Society of Gene Therapy: Abstracts for presentation at 2001 annual meeting.

RESEARCH GRANT AWARDS

Saswati Chatterjee, Ph.D.

- 1/92-12/92** City of Hope Cancer Center Seed Grant Award.
AAV vector-mediated gene transfer into primary peripheral blood leukocytes and bone marrow cells. S. Chatterjee, Principal Investigator.
- 1/92-12/92** City of Hope Biomedical Research Support Grant.
A comparison of transduction efficiencies of adeno-associated virus-based vector with retroviral vectors. S. Chatterjee, Principal Investigator.
- 10/92-9/93** City of Hope Cancer Center Seed Grant Award.
Construction and use of an adeno-associated virus vector encoding the MDR-1 gene. S. Chatterjee, Principal Investigator.
- 10/92-9/96** 1P01CA 59308. **Gene Therapy of Marrow Cells.** J. Zaia, Principal Investigator.
National Cancer Institute, NIH, Gene Therapy Program, Program Project Grant:
Project 1: **AAV-mediated gene transfer into hemopoietic cells.** S. Chatterjee, Project Leader.

Project 2: **AAV vector safety and regulatory issues.** S. Chatterjee, Co-investigator.
- 9/96-9/97** No cost extension of CA59308.
- 11/92-10/95** 3U01AI25959. J. Zaia PI. National Institute of Allergy and Infectious Diseases, NIH; National Cooperative Drug Discovery Grants (NCDDG) for the treatment of HIV Infection.
Project: **Anti-SIV gene therapy in Rhesus Monkeys.** S. Chatterjee, Co-Investigator.
- 7/96-5/99** 1R01AI40001. **Multivalent AAV vectors for HIV-1 gene therapy.** S. Chatterjee, Principal Investigator. National Institute of Allergy and Infectious Diseases, NIH. \$540,014.
- 6/99-5/00** No cost extension of AI40001.
- 9/97-9/00** 1R01CA75186. **Gene modified dendritic cells for tumor immunotherapy.** S. Chatterjee, Principal Investigator. National Cancer Institute, NIH. \$685,475.
- 10/00-9/02** No cost extension of CA75186.
- 7/99-6/04** 1P01HL60898-01A1. **Gene therapy approaches for blood and vascular diseases.** KK Wong, Principal Investigator. National Heart, Lung, Blood Institute, NIH. \$7,262,996

Project 1: AAV Transduction of quiescent hematopoietic stem cells. S. Chatterjee, Project leader. \$1,040,014 direct cost.

Core B: Vector Core. S. Chatterjee, Core leader. \$928,159 direct cost.
- 4/00-3/03** 1P01CA30206-19. Bone Marrow Transplantation for Hematologic malignancies. SJ Forman, Principal Investigator. Project IV. S. Chatterjee, Co-Investigator. \$214,859 direct cost.

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- 9/99-8/04 NIAID-DAIT-BAA-99-12.. Clinical Trials and Clinical markers of Immunologic Diseases. H. Oppenshaw, Principal Investigator; S. Chatterjee, Co-Investigator.
- 10/99-9/04 NIAID-DAIT-BAA-99-31. National Collaborative Study of Stem Cell Transplanatation for Autoimmune Disease. H. Oppenshaw, Principal Investigator; S. Chatterjee, Co-Investigator.

PATENTS & LICENSES

- 12/12/95 US Patent No.: 5,474,935. Saswati Chatterjee & K.K. Wong Jr.: **Adeno-associated virus (AAV)-based eukaryotic vectors.**
- 6/95 **AAV packaging cell lines.** Licensed by Applied Immune Sciences, San Jose, CA.
- 12/97 **Adeno-associated virus (AAV)-based eukaryotic vectors & AAV packaging cell lines.** Licensed by Strata Biosciences, Alameda, CA.

BIBLIOGRAPHY

1. **Chatterjee, S.** and Hechtman, P.: t-aminobutyric acid metabolism in brain homogenates of the spastic mouse. Biochem.Gen. 15: 1147-151, 1977.
2. **Chatterjee S.:** Placental H-2 antigens and changes in the maternal lymphoid system during allogeneic pregnancy in the mouse. M.Sc. Thesis, McGill University, 1978.
3. **Chatterjee, S.,** and Lala, P.K.: The localization of H-2 antigens on mouse trophoblast cells. J. Exp. Med. 149: 1238-1253, 1979.
4. **Chatterjee, S.,** Santer, V., and Lala, P.K.: Changes in maternal small lymphocyte subsets during allogeneic pregnancy in the mouse. Cell. Immunol. 50: 290-304, 1980.
5. **Chatterjee, S.** and Lala, P.K.: MHC antigens on mouse trophoblast cells: absence of Ia antigens despite the presence of H-2K and D. J. Immunol. 127: 2070-2073, 1981.
6. **Chatterjee S.,** and Lala, P.: Localization of paternal H-2K antigens on murine trophoblast cells in vivo. J. Exp. Med. 155: 1679-1689, 1982.
7. **Chatterjee, S.:** The immunobiology of feto-maternal relationship. Ph.D. Thesis, McGill University, 1982.
8. **Chatterjee, S.,** Montgomery, B., and Lala, P.K.: Alloantigenicity of trophoblast cells. Amer. J. Reprod. Immunol. 3: 127-131, 1983.
9. Lala, P.K., **Chatterjee, S.,** and Montgomery, B.: Major histocompatibility antigens on murine and human

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- trophoblast cells. Transplant. Proc. 15: 883-886, 1983.
10. Lala, P.K., **Chatterjee, S.**, Kearns, M., Montgomery, B., and Colavincenzo, V. Immunobiology of the Feto-Maternal Interface. Immunol. Rev. 75: 87-116, 1983.
 11. **Chatterjee, S.**, Parhar, R. and Lala, P.K.: An Evaluation of the Maternal Natural Killer Cell Population during the course of Murine Pregnancy. Cell Immunol. 84: 264-275, 1984.
 12. Bluestone, J.A., Potter, T.A., **Chatterjee, S.**, and Rajan, T.V.: CTL recognize different determinants from those defined serologically on L^d somatic cell mutants. J.Immunol. 133:1168-1173, 1984.
 13. **Chatterjee S.**, Berg, S.K. and Sachs, D.H.: Molecular and serologic analysis of products of the D-region of H-2^a. Transplant. Proc. 17: 722-724, 1984.
 14. **Chatterjee, S.**, Schlauder, G., Sachs, D.H., Glimcher, L.H., Paul, W.E., and McKean, D.J.: A biochemical analysis of I-A^k molecules from mutant antigen presenting cell lines. Immunogenetics 23: 121-125, 1986.
 15. **Chatterjee, S.**, Lillehoj, E., Hernandez, D.M., Coligan, J.E. and Sachs, D.H.: Analysis of the D-region products of H-2q using monoclonal antibodies reveals the expression of a new class 1-like molecule. Immunogenetics 25: 7-14, 1987.
 16. Rabinowitz, R., Sharrow, S.O., **Chatterjee, S.**, Rogers, M.J., and Sachs, D.H.: Qa alloantigen expression on functional T lymphocytes from spleen and thymus. Immunogenetics 24: 391-401, 1987.
 17. **Chatterjee, S.**, Wong K.K., Rose J.A. and Johnson, P.R. : Transduction of intracellular resistance to HIV production by an adeno-associated virus-based antisense vector. In Vaccine 91: Modern approaches to new vaccines including the prevention of AIDS. R.M. Channock, H.S. Ginsberg, F. Brown and R.A.Lerner Eds. Pp 85-90. Cold Spring Harbor Laboratory Press. 1991.
 18. Wong K.K., Rose J.A. and **Chatterjee, S.**: Restriction of HSV-1 production in cell lines transduced with an antisense viral vector targeting the ICP4 gene. In Vaccine 91: Modern approaches to new vaccines including the prevention of AIDS. R.M. Channock, H.S. Ginsberg, F. Brown and R.A.Lerner Eds. Pp 183-189. Cold Spring Harbor Laboratory Press. 1991.
 19. Wong K.K. and **Chatterjee, S.**: Controlling herpes simplex virus (HSV) infections: Intracellular immunization, the way of the future? Current Topics in Microbiology and Immunology, B. Rouse, Editor. Springer Verlag 179: 159-174, 1992.
 20. **Chatterjee, S.**, Johnson, P.R. and Wong, K.K.: Dual target inhibition of HIV-1 in vitro with an adeno-associated virus-based antisense vector. Science, 258: 1485-1488, 1992.
 21. Zaia, J.A, **Chatterjee, S.**, Wong, K.K., Elkins, D., Taylor, N.R. and Rossi, J.J.: Status of ribozyme and antisense-based developmental approaches for anti-HIV-1 therapy. In Antisense Strategies. The Annals of the New York Academy of Sciences, 660: 95-106, 1992.
 22. **Chatterjee, S.** and Wong, K.K. Adeno-associated viral vectors for the delivery of antisense RNA. Methods: A Companion to Methods in Enzymology. 5: 51-59, 1993.

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23. Podsakoff GM, Wong KK and **Chatterjee, S.** Stable and efficient gene transfer into non-dividing cells by adeno-associated virus-based vectors. J. Virol. 68: 5656-5666, 1994.
24. Lu, D, **Chatterjee S**, Brar, D, and Wong KK. Ribozyme-mediated in vitro cleavage of transcripts arising from the major transforming genes of human papillomavirus type 16. Cancer Gene Therapy, 1:267-277, 1994.
25. **Chatterjee S**, Wong KK, Lu D, Permana PA and Podsakoff GM. Novel approaches for efficient gene transfer into hematopoietic progenitor cells: the use of adeno-associated virus vectors. Bone Marrow Transplantation, 15: S309-S313, 1995.
26. **Chatterjee S**, Lu D, Podsakoff GM, Wong, KK. Strategies for efficient gene transfer into hematopoietic cells: The use of adeno-associated virus vectors in gene therapy. The Annals of the New York Academy of Sciences. 770: 79-90, 1995.
27. Shaughnessy, E, Lu D, **Chatterjee S**, and Wong, KK. Parvoviral vectors for the Gene Therapy of Cancer. Seminars in Oncology 23: 159-171, 1995.
28. **Chatterjee S** and Wong, KK. Adeno-associated virus vectors for gene therapy of the hematopoietic system. Current Topics in Microbiology and Immunology., Giraud and Berns Ed. Springer Verlag 218: 61-73, 1996.
29. Wong KK and **Chatterjee S**. Adeno-associated virus vectors as antivirals. Current Topics in Microbiology and Immunology. Giraud and Berns Ed. Springer Verlag 218: 145-170, 1996.
30. Fisher-Adams G, Wong, KK, Podsakoff GM, Forman, SJ and **Chatterjee S**. Integration of adeno-associated virus vector genomes in Human CD34 cells following transduction. Blood, 88: 492-504, 1996.
31. Bertrand E, Castanotto D, Zhou C, Carbonelle C, Lee NS, Good P, **Chatterjee S**, Grange T, Pictet R, Kohn D, Engelke D and Rossi J. The expression cassette determines the functional activity of ribozymes in mammalian cells by controlling their intracellular localization. RNA, 3: 75-88, 1997.
32. Margolin K, Negrin RS, Wong KK, **Chatterjee S** and Forman SJ. Cellular immunotherapy and autologous transplantation for hematologic malignancies. Immunol. Rev. 157: 231-240, 1997.
33. Wong KK, Shaughnessy E, Lu D, Fisher-Adams G and **Chatterjee S**. Parvovirus Vectors for the Gene Therapy of Cancer. In Gene Therapy of Cancer. E. Lattime and S. Gerson Eds. Academic Press. 1998.
34. **Chatterjee S**, Li W, Wong C, Lu D, Fisher-Adams G, Guha M, Macer J, Forman SJ and Wong KK. Transduction of primitive human marrow and cord blood-derived hematopoietic progenitor cells with adeno-associated virus vector. Blood. 93:1882-1894, 1999.
35. Mi J, **Chatterjee S**, Wong KK, Forbes C, Lawless G, Tobin AJ. Recombinant adeno-associated virus drives constitutive production of glutamate decarboxylase in neural cell lines. J Neuroscience Research, 57: 137-149, 1999.
36. **Chatterjee S** and Wong KK. Adeno-associated virus vectors for the delivery of ribozymes. In "Intracellular

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Ribozyme Applications: Principles and Protocols." JJ Rossi and L Couture Eds. pp189-215, Horizon Scientific Press. 2000.

37. Wong KK and **Chatterjee S**. Parvovirus Vectors for the Gene Therapy of Cancer. In Gene Therapy of Cancer. Second Edition, E. Lattime and S. Gerson Eds. Academic Press. 2001. *In Press*.
38. Sun J, Krouse RS, Forman SJ, Senitzer D, Sniecinski I, **Chatterjee S** and K.K. Wong Jr. Immunogenicity of a P210BCR-ABL Fusion Domain Candidate DNA Vaccine Targeted to Dendritic Cells by an rAAV Vector In Vitro. Blood. *In Review*.
37. Wong CA, Li W, Forman SJ, Wong KK, **Chatterjee S**. Gene transfer into quiescent CD34+CD38- hematopoietic progenitor cells in G0 with adeno-associated virus vectors. *In preparation*.
38. Fisher-Adams G, Wong KK and **Chatterjee S**. Long term transcriptional potentials of genes encoded by integrated AAV vectors in transduced cell lines. *In preparation*.
39. Li LJ, Brar D, Permana P, Rossi JJ, Wong KK and **Chatterjee S**. Promoter strengths and orientation dependance of transgene expression from multivalent AAV vectors. *In preparation*.
40. Li LJ, Rossi JJ, Forman SJ, Wong KK and **Chatterjee S**. Long term inhibition of HIV-1 replication in progeny macrophages derived from CD34+ hematopoietic progenitor cells transduced with AAV vectors encoding anti-HIV genes. *In preparation*.
41. Wong KK, Rosborough E, Aye T and **Chatterjee S**. Permanent packaging cell lines for the production of adeno-associated virus vectors. *In preparation*.

ABSTRACTS & PRESENTATIONS.

1. **Chatterjee, S.**: Localization of major histocompatibility antigens on mouse trophoblast cells. Anat. Rec. 193: 502-503, 1979.
2. **Chatterjee, S.** and Lala, P.K.: Localization of mouse trophoblast cells. Fed. Proc. 38:929,1979.
3. **Chatterjee, S.**, and Lala, P.K.: Characterization of maternal small lymphocyte subsets during allogeneic pregnancy. Proc. Can. Fed. Biol. Soc. 1979.
4. **Chatterjee, S.**, and Lala, P.K.: Ia antigens on mouse trophoblast cells. Proc. Can. Fed. Biol. Soc., 1980.
5. **Chatterjee, S.**, and Lala, P.K.: MHC antigens on mouse trophoblast cells. Presented at the Fourth International Congress of Immunology, Paris and 7th International Convocation on Immunology: Immunobiology of the Major Histocompatibility Complex, Buffalo, NY, USA, 1980.
6. **Chatterjee, S.**, and Lala, P.K.: Surface marker analysis of maternal T lymphocyte subsets during pregnancy. International Conference on Reproductive Immunology, Banff, Alberta, Canada. J.Reprod.Immunol., Suppl. S25, 1981.

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7. Chatterjee, S., and Lala, P.K.: Natural killer cell activity during murine pregnancy. International Conference on Reproductive Immunology, Banff, Alberta, Canada. J.Reprod. Immunol. Suppl. S24, 1981.
8. Chatterjee, S., and Lala, P.K.: Surface marker characterization of maternal T lymphocyte subsets during pregnancy. Proc. Can. Fed. Biol. Soc. 24: 140, 1981.
9. Chatterjee, S., and Lala, P.K.: In situ localization of H-2K antigens. Fed. Proc. 41(3): 426, 1982.
10. Chatterjee, S., and Lala, P.K.: In vivo localization of H-2K antigens. Fed. Proc. 41(3): 426, 1982.
11. Lala, P.K., and Chatterjee, S.: A comparison of host natural killer cell responses during tumour-bearing and pregnancy. Proc. Can. Fed. Biol. Soc. 24: 140, 1982.
12. Chatterjee, S., and Lala, P.K.: In situ localization of paternal H-2K antigens on trophoblast cells. Amer. J. Reprod. Immunol. 2(3): 170, 1982.
13. Lala, P.K., Chatterjee, S., and Montgomery, B.: Major histocompatibility antigens on murine and human trophoblast cells. Proc. IX Intl. Congress Transplant. Soc., Brighton, U.K., 1982.
14. Chatterjee, S., Hernandez, D.M., and Sachs, D.H.: An immunochemical analysis of the products of the D region of H-2^d. Fed. Proc. 42: 1370, 1983.
15. Glimcher, L., Chatterjee, S., Schlauder, G., Paul, W.E., and McKean, D.J.: Functional Ia mutant antigen presenting cell lines. Fed. Proc. 43: 1733, 1984.
16. Schlauder, G., Beck, B., Bell, M., Chase, C., Chatterjee, S., Glimcher, L., Paul, W., Pierres, M., Sachs, D. H., and McKean, D.J.: Biochemical characterization of mutant I-A^k molecules from antigen presenting B cell - B lymphoma hybridomas. Fed. Proc. 43: 1599, 1984.
17. Chatterjee, S., Hernandez, D.M. and Sachs, D.H.: An analysis of the antigens encoded by the D - region of H-2^d. 10th Ann. Cong. Transplant. Soc., Minneapolis, 1984.
18. Rogers, M.J., Chatterjee, S. and Siwarski, D.F.: Sequences of class I genes from Mus Pahari: implications for the evolution of a multi-gene family. Prog. in Immunol. VI. 1986.
19. Chatterjee, S., Sebring, E., Rose, J.: AAV virions contain genomes that are processed at or near the DNA origin. Presented at the American Society for Virology, June 12-16, 1988, Austin, Texas.
20. Chatterjee, S., Wong, K.K. Jr., Rose, J.A. and Johnson, P.R.: Establishment of intracellular resistance to human immunodeficiency virus replication. Cold Spring Harbor Meeting: RNA Tumour Viruses, NY., May 23-25, 1990.
21. Chatterjee, S., Johnson, P.R. and Wong, K.K. Jr.: Transduction of intracellular resistance to HIV production by an adeno-associated virus-based antisense vector. Cold Spring Harbor Meeting: Modern Approaches to New Vaccines Including the Prevention of AIDS, NY, September 12-16, 1990.

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22. Wong, K.K., Rose, J., **Chatterjee, S.**: Restriction of HSV-1 replication in cell lines transduced with an antisense viral vector targeting the HSV-1 ICP4 gene. Cold Spring Harbor Meeting: Modern Approaches to New Vaccines, NY, September 12-16, 1990.
23. **Chatterjee, S.** and Wong, K.K. : Transduction of intracellular resistance to virus replication using adeno-associated virus-based vectors. The Second International Conference on Catalytic RNA as anti-HIV agents: Design and Delivery, San Diego, CA, October 21-24, 1990.
24. Wong, K.K., Rose J. A. and **Chatterjee, S.**: Inhibition of herpes simplex virus type 1 (HSV-1) production in cell lines transduced with an adeno-associated virus based antisense vector targeting the requisite ICP4 gene. The Second International Conference on Catalytic RNA as anti-HIV agents: Design and Delivery, San Diego, CA, October 21-24, 1990.
25. KK Wong and **S Chatterjee**. Establishment of intracellular resistance to HSV-1 production in cells transduced with an adeno-associated virus based antisense vector. J. Cell. Biochem. Supp 16F: 52, 1992.
26. **Chatterjee, S.**, and Wong, KK.: Transduction of intracellular resistance to HIV by an adeno-associated virus-based antisense vector. J. Cell. Biochem. Supp 16F: 58, 1992.
27. **Chatterjee, S.**, Forman, S., Zaia, J., Wong, K.K.: An adeno-associated virus-based dual-target antisense vector: High efficiency inhibition of HIV-1 and transduction of primary hemopoietic cells. Cold Spring Harbor Meeting: Gene Therapy, NY, September 22-26, 1992.
28. Wong, K.K. and **Chatterjee, S.** Transduction of intracellular resistance against HIV utilizing an adeno-associated virus-based vector: potential for antiviral gene therapy. 32nd Annual ICAAC Meeting, Anaheim, October 11-14, 1992.
29. **Chatterjee, S.**, Wong, K.K., Podsakoff, G., Zaia, J., Forman, S. Adeno-associated virus vectors for high efficiency gene transfer into primary human hematopoietic cells. American Society of Hematology. Blood 80: Suppl 1, 167A, 1992.
30. **Chatterjee, S.**, Podsakoff, G., Wong K.K. Strategies for antiviral gene therapy: Use of an Adeno-associated virus (AAV)-based vector system to confer intracellular resistance to targeted viruses. Third International Symposium on catalytic RNAs (ribozymes) and targeted gene therapy for the treatment of HIV infection. Invited talk at San Diego, December 6-11, 1992.
31. **Chatterjee S**, Podsakoff, G.M. and Wong K.K.: A Gene Therapeutic Approach to AIDS: Adeno-Associated Virus Vectors for the Delivery of Genes Encoding Antisense RNA and Ribozymes Targeting HIV-1. Invited talk for Coordinated Therapies for HIV-1 Infection. Washington DC, July 1993.
32. **Chatterjee S**, Podsakoff, G.M. and Wong K.K.: Adeno-associated virus vectors for the delivery of anti-HIV genes. Invited talk for Symposium on Genetic Therapies for HIV-1 Infection. , p 441, 33rd ICAAC Meeting, New Orleans, LA, October, 1993.
33. Wong, K.K., Podsakoff, G., Lu, D. and **Chatterjee, S.** High efficiency gene transfer into growth arrested cells utilizing an adeno-associated virus-based vector. Blood 82, Suppl.1, 302a, 1993

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34. Snyder, D., Wu Y., Wong, K.K., **Chatterjee, S.** and Forman, S.J. Ribozyme-viral vector constructs inhibit BCR-ABL gene expression in Philadelphia-chromosome positive cell lines. Blood 82, Suppl.1, 40, 1993.
35. Shaughnessy, E., Wong, K.K. and **Chatterjee, S.** Adeno-associated virus-based vectors: Biology and potential role in the gene therapy of cancer. Cancer Gene Therapy, 1, Suppl. 1, 9, 1993.
36. **Chatterjee S** Adeno-associated virus: a high efficiency vector for gene therapy. Invited talk for Keystone Symposia on Controversies on Bone Marrow Transplantation. January, 1994.
37. **Chatterjee S**, Wong KK, Podsakoff G, Lu D, Permana P and Brar D. Strategies for anti-HIV gene therapy: the use of adeno-associated virus vectors. Invited talk for UCLA AIDS Symposium, Palm Springs, CA, March 3-6, 1994.
38. Permana P, Wong KK, Brar D and **Chatterjee S.** Transcription of anti-HIV RNA from adeno-associated virus vectors encoding RNA polymerase II- and III-dependent promoters. Poster session. UCLA AIDS Symposium, Palm Springs, CA, March 3-6, 1994.
39. Shaughnessy, E., Wong, K.K., Podsakoff, G, Kane, S and **Chatterjee, S.** Adeno-associated virus vectors for MDR-1 gene therapy. Proc. Amer. Assoc. for Cancer Research 35, 373, 1994.
40. Lu, D, **Chatterjee, S**, Brar, D and Wong KK. High efficiency in vitro cleavage of transcripts arising from the major transforming genes of human papillomavirus type 16 mediated by ribozymes transcribed from an adeno-associated virus vector. Proc. Amer. Assoc. for Cancer Research 35, 309, 1994.
41. **Chatterjee S**, Wong KK Jr, Podsakoff G, Shaughnessy E. Gene transfer into human hematopoietic progenitor cells by adeno-associated virus vectors for the treatment of malignancies. Third International Conference on the Gene Therapy of Cancer, Coronado, CA; November 10-12; Cancer Gene Therapy 1:323 (III-59), 1994.
42. Brar D, Wong KK Jr, Permana P, **Chatterjee S.** Promoter interactions in adeno-associated virus vectors encoding multiple gene cassettes: potential use in anti-oncogene vector design. Third International Conference on the Gene Therapy of Cancer, Coronado, CA; November 10-12, Cancer Gene Therapy 1:321 (V-53), 1994.
43. Wong KK, Brar D, Rossi J, **Chatterjee, S.** Primary human CD34+ peripheral blood stem cells (PBSCs) are efficient targets for adeno-associated virus vector-mediated gene transfer: Prospects for anti-AIDS genetic intervention. Blood, 84, Supplement 1, 743a; Nov 15, 1994. American Society of Hematology Meeting December 2-6, 1994, Nashville, Tenn. No.2957.
44. Podsakoff G, Shaughnessy EA, Lu D, Wong KK Jr, **Chatterjee S.** Long term in vivo reconstitution with murine marrow cells transduced with an adeno-associated virus vector. Blood, 84, Supplement 1, p. 256a; Nov 15, 1994. American Society of Hematology Meeting December 2-6, 1994, Nashville, Tenn. No.1009
45. **Chatterjee S**, Podsakoff G, Wong KK Jr. Gene transfer into terminally differentiated primary human peripheral blood-derived mononuclear cells by adeno-associated virus. Blood, 84, Supplement 1, p360a; Nov 15, 1994. American Society of Hematology Meeting December 2-6, 1994, Nashville, Tenn. No.1424.

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MAJOR INVITED TALKS

The Third International Symposium on Catalytic RNAs and Targeted Gene Therapy for the treatment of HIV infection. San Diego, December 6-11, 1992.

Coordinated Therapies for HIV-1 Infection. Washington DC, July 11-16, 1993.

Symposium on Genetic Therapies for HIV-1 Infection. American Society for Microbiology, 33rd ICAAC Meeting, New Orleans October, 1993.

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Keystone Symposia: Controversies on Bone Marrow Transplantation. Keystone, CO, January 23-30, 1994.

UCLA AIDS Symposium, Palm Springs, CA, March 3-6, 1994.

Symposium on Stem Cells: Prospects for the Clinic. J Mule & J Larrick, organizers. Palo Alto, CA, February 6-7, 1995.

New York Academy of Sciences Symposium on Bone Marrow Transplantation: Foundations for the 21st century. Orlando, FL, March 15-18, 1995. Robert Sackstein, organizer.

Hemopoietic Stem Cell Gene Therapy. Chevy Chase, MD. September 28-October 1, 1995. G. Stamatoyanopoulos, organizer.

AAV vectors: Gene transfer into quiescent cells. Bethesda, MD December 6, 1995. C. McKeon, RJ Samulski, organizers NIDDK, NIH.

AAV vectors for Gene Transfer into Stem Cells. Invited presentation to the Scientific Board of Systemix, Palo Alto CA, March 8, 1996.

FDA Gene Therapy Conference. July 11-12, 1996. Robert Anderson, Center for Biologics Evaluation and Research, FDA & Biological Resources Branch, NCI, NIH, organizer.

American Society of Hematology 1996 annual meeting. Moderator, oral presentation session on "Gene Therapy: Gene Transfer and Biology".

Annual Symposium on Gene Medicine. June 13, 1997. William McBride and James Economou, organizers. UCLA, Los Angeles, CA.

Principal Investigators Meeting, NHLBI, NIH, Rockville MD, July 6-7, 2000. Sonia Skarlatos, organizer.

Symposium on Cardiovascular Gene Therapy 2000. September 21, 2000. PK Shah & T. Rajavashisth, organizers, Cedars Sinai Medical Center.

Annual Meeting of the American Society of Gene Therapy. Workshop on AAV vectors.. rAAV vectors in vivo. May 2001, Seattle, WA.

Analysis of CD34 cells subpopulations from OM. Shows that CD34+CD38- cells are in G0.

FIGURE 1

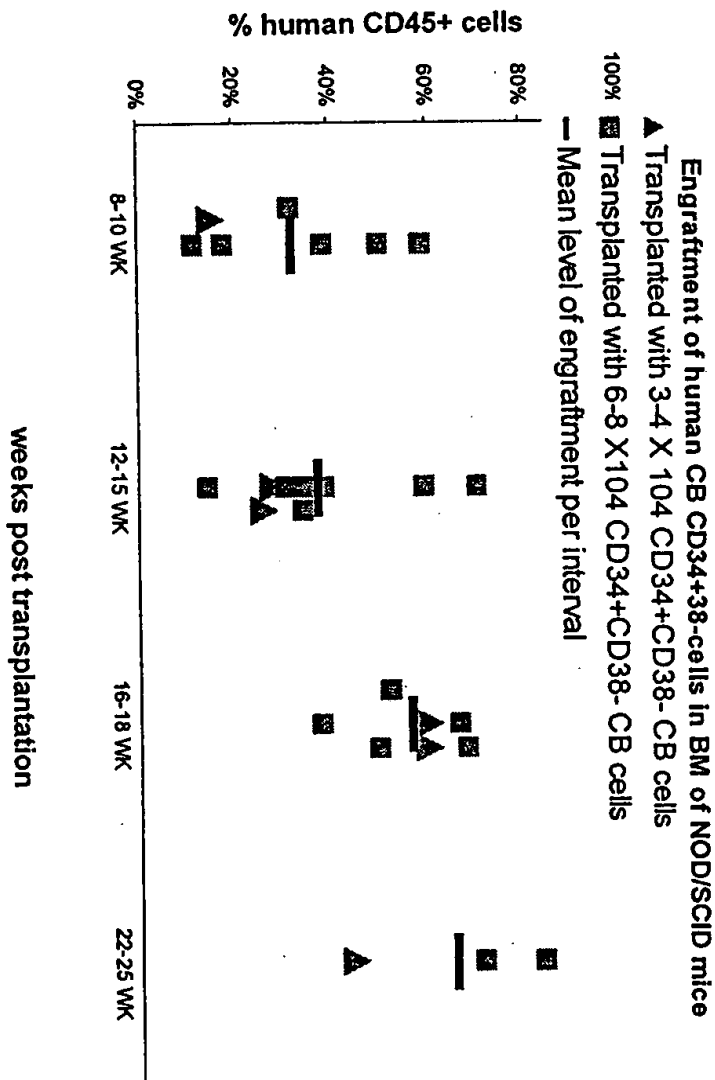


FIGURE 2

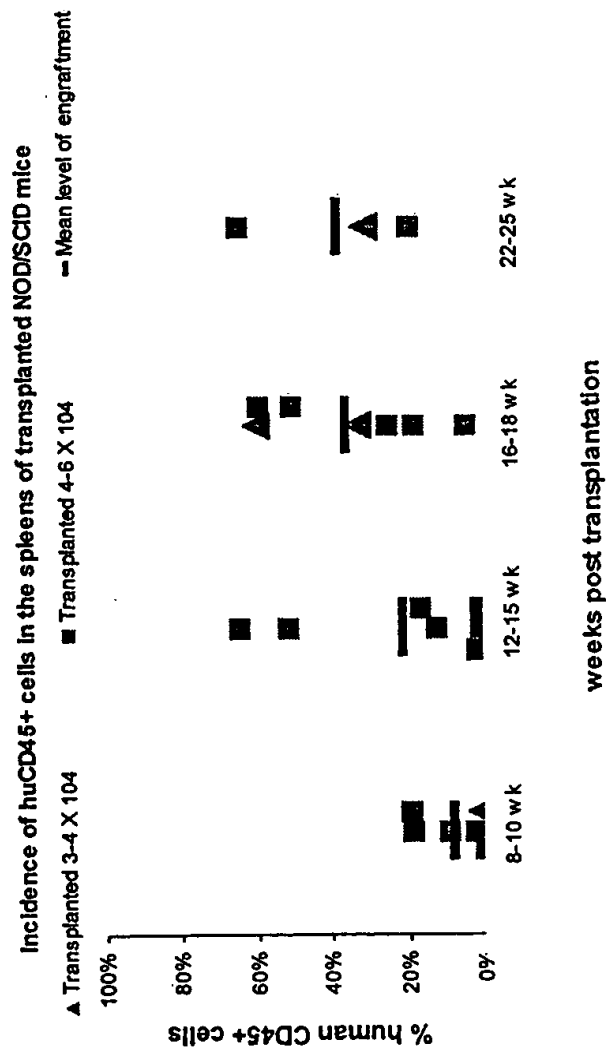


FIGURE 3

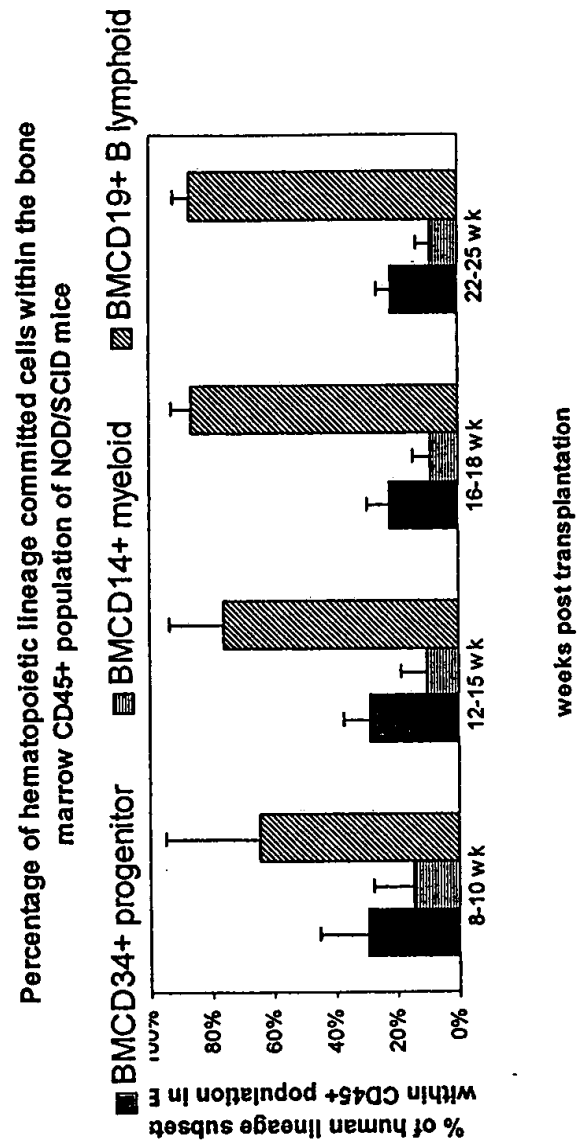
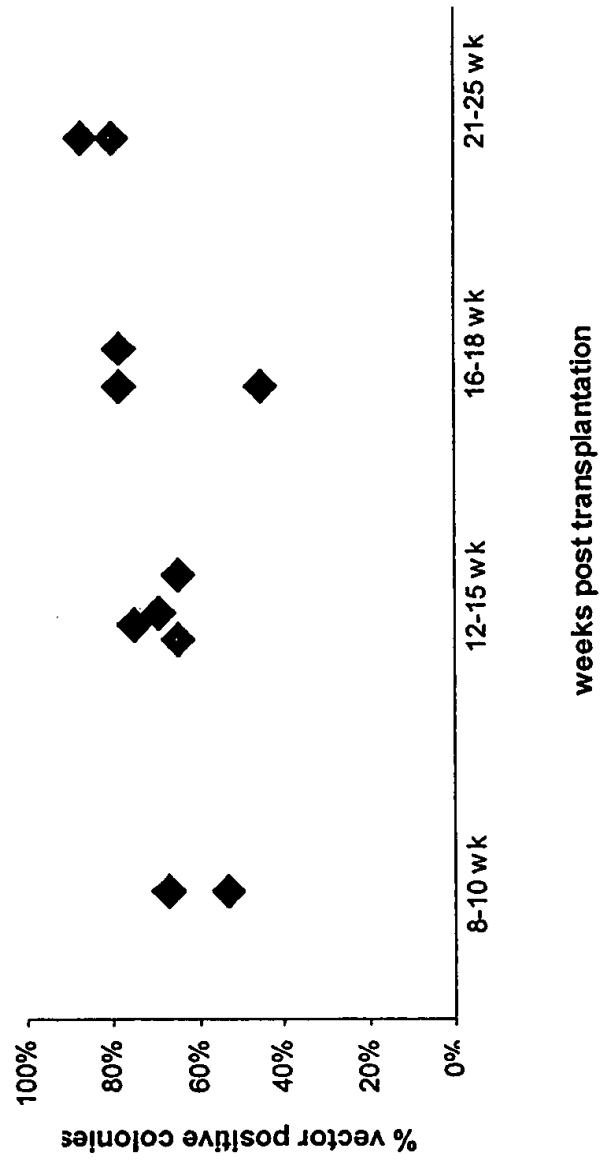


TABLE 1

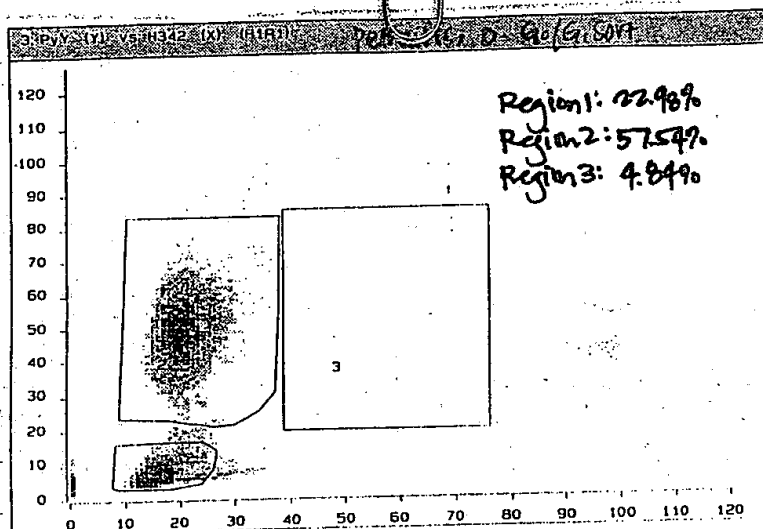
<u>Weeks Post Transplant</u>	<u>CD34+</u>	<u>Bone Marrow CD14+</u>	<u>CD19+</u>	<u>Spleen CD19+</u>
9-10	75% (3/4)	100% (4/4)	50% (2/4)	25% (1/4)
12-15	57% (4/7)	50% (3/6)	50% (3/6)	67% (4/5)
17-18	43% (3/7)	57% (4/7)	57% (4/7)	43% (3/7)
22-25	75% (3/4)	67% (2/3)	33% (1/3)	33% (1/3)
Total	59% (13/22)	65% (11/17)	53% (9/17)	53% (9/17)

FIGURE 4



cont'd

Stained the rest of the Cp34+ cells w/ Hoechst & Pyronin
to sort for G₀ and G₁ populations.



Bone Marrow sample today! 15 mL

98-24-19 4
ATESHKADI, ARASH
H / A 03/21/1968

Mononuclear cells = 1.3×10^6

Total Cp34+ = 2.8×10^6 ~ 2.2%

Stored mononuclear cells @ 4°C in 50% FCS

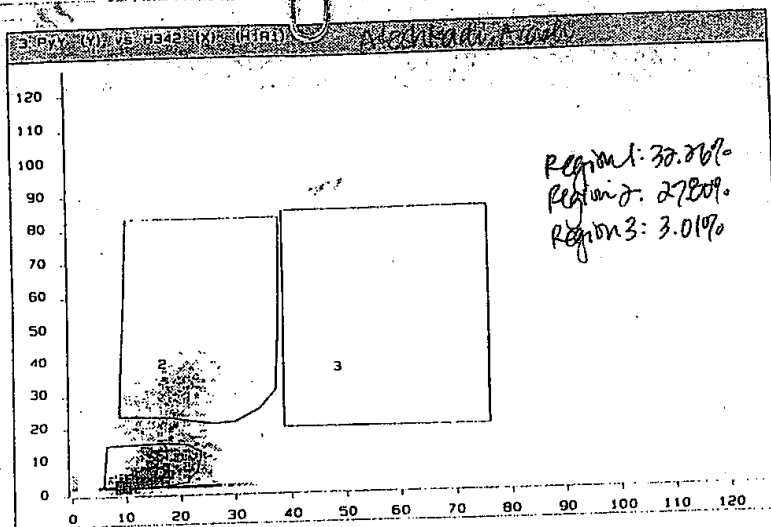
Isolated Cp34+ from mononuclear cells today.

Labeled → Ran through the column.

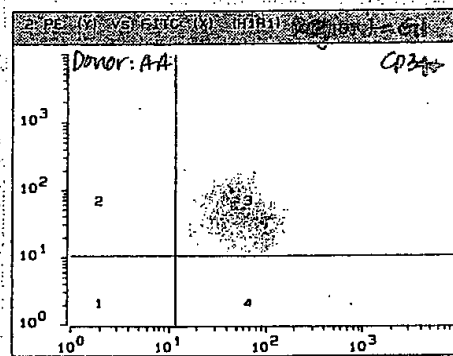
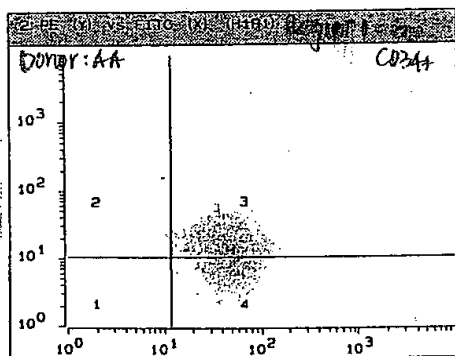
Total Cp34+ count = 2.8×10^6 cells.

Store @ 4°C in 50% FCS → Sort @ 6, tomorrow!

: G₀/G₁ Sort: using same [I] of Hoechst / Pyronin as usual.



performed CD34+ CD38- Ab stain on cells sorted in Region 1 and Region 2.



Sort problems:

The cells gated for Region 1, 2 & 3 ~~were~~ shifted out →
∴ the cells analyzed were not the entire population.

CD34+CD38- Analysis.

Although there seems to be 2 diff. pop. of cells... I expected more

Donor/(Week#)	(+)JMP	(-)JMP	% +MP	IP (<3)	IP (>3)	(-)IP	% +IP (<3)	% +IP (>3)
Ateshkadi, A. 6/11								
<u>CYTOKINES</u>								
AA G0 CsCl (4)	2	22	8.3	39	37	206	13.8	13.1
AA G0 CsCl (6)	6	32	15.8	15	39	198	6.0	15.5
AA G0 CsCl (8)	4	28	12.5	20	35	200	7.8	13.7

80°C 2hr
 after @ 42°C for 5 hr
 0.75 λ (4.15 μ)

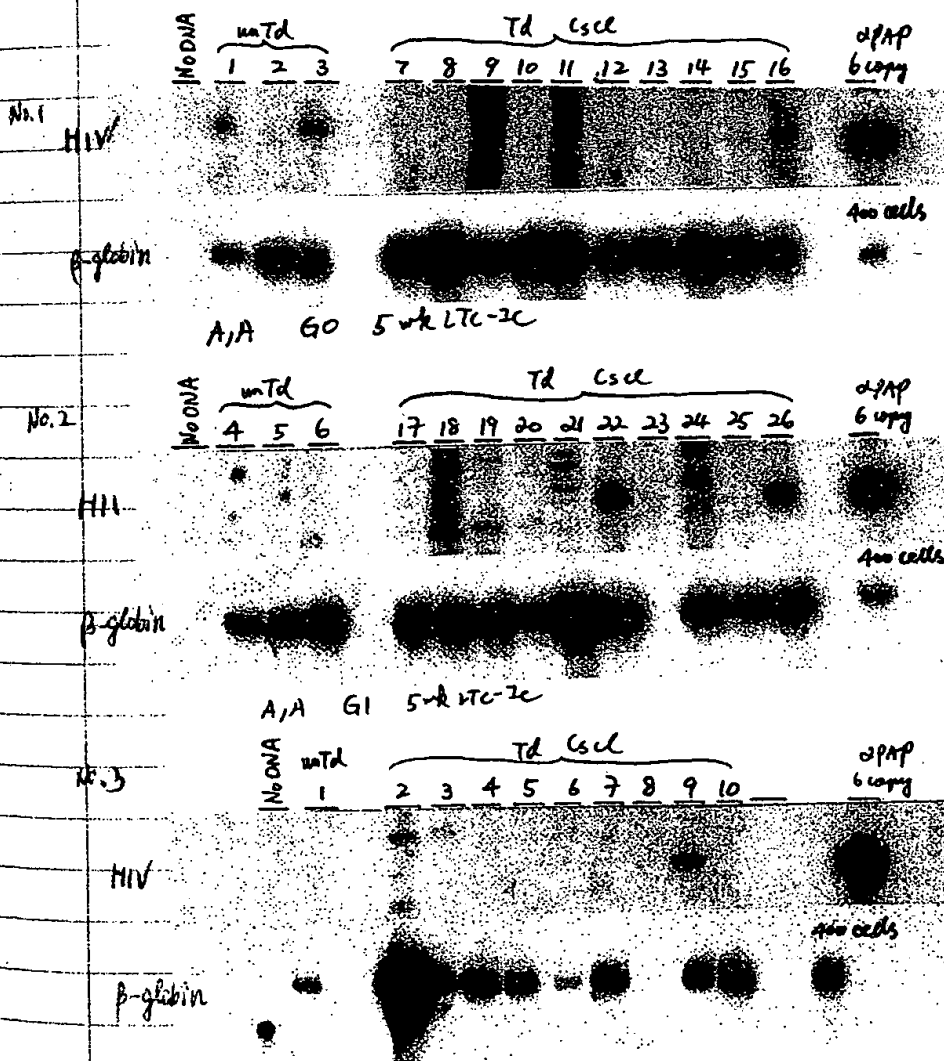
* Wash blot from
 65°C 2 x 15' 0.5XSSC, 0.1% SDS

* Precipitate down PCR products (and)
 -70°C 1 μ l glycogen/sample

Volume Total counts per
 50 112 x 10⁶

* Run hot PCR products () on 5% Urea/polyacrylamide Gel
 A, A GO 5 wk LTC-2c

per w/in 2 blots



Load 1/3 of HIV, 1/2 of β -globin, 1/5 of positive control

400V

8°C dry 2hr

* Pick up colonies

MERCADO, ORFEDALIA ()

5 wk LTC-2C CD34+CD38-

count colonies: untd 157 (all CFU-GM except 9)

Td 762 (all CFU-GM except 61)

All colonies are big and nice.

pick up colonies:

For PCR: untd: 1-4 (1-2: CFU-GM

3-4: BFU-E)

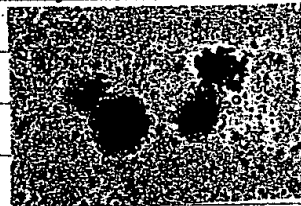
Td: 5-24 (5-21: CFU-GM

22-24: BFU-E)

For PCR: untd: 25 (CFU-GM)

Td: 26-34 (26-32: CFU-GM

33-34: BFU-E)

150 μ l STE, 0.5 μ l RNase-DNase 37°C 13.75 μ l 20% SDS, 2.5 μ l PK (10mg/ml) 37°C 1red
colony

Mature BFU-E (Day 10)

(3-8 multi-clustered)

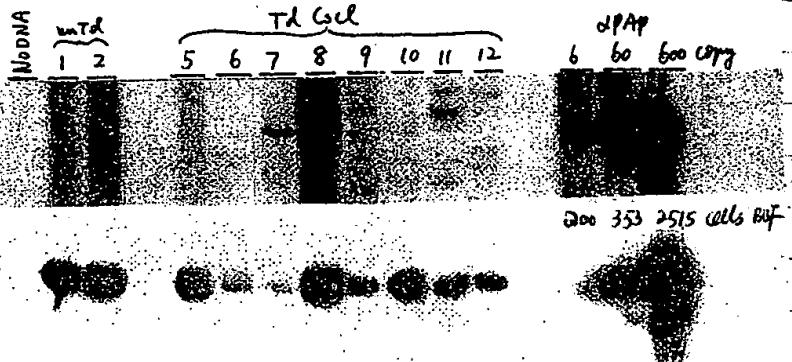


Primitive BFU-E (Day 18)

(9 or more multi-clustered)

* Run hot PCR products () on 5% Urea/Polyacrylamide Gel.

A, A 60 8 wk LTC-2C



A, A ¹⁰ GO 8wK LTC-2c

[illegible]

6-60-600 copy

No. 2

384 + CD38- (2)
all CFU-GM except 9 BFu-E
1 CFU-GM except 61 BFu-E
big and nice.

200 53 255 cells

No. 2

A, A GI 8 wk LTC-2c

No DNA unit Td Cscl
1 2 3 5 6 7 8 9 10 11

αPAP
6 60 600 6000

No. 3

1-4 (1-2: CFu-GM
3-4: BFu-E)
5-24 (5-21: CFu-GM
22-24: BFu-E)

No. 3

25 (CFU-GM)
26-34 (26-32: CFU-GM
33-34: BFC-E)

ul RNase - DNase 37°C 1 hr
2.5 ul PK (10mg/ml) 37°C 0/N

1. 5% Urea / Polyacrylamide

load 13

~~total 45~~

400 ✓
8°C day 2 hr

12

2PAP
6 60 600 copy

200 353 2515 cells Baf

pick up colony. G0: un 1-4 (CFU-GM)

Td 5-24

G1: un 1-3 (CFU-GM)

4 (cluster on stroma)

Td 5 (colony-cluster CFU-GM)

6-7 (cluster floating)

8-15 (cluster loosely on stroma)

150 μ l STE, 0.5 μ l RNase PNB free 37°C 1 hr

3.75 μ l 20% SDS, 2.5 μ l PK (1000 μ l) 37°C 9h

10/91

G0/91

c, 1/2 back to culture to me

Run left PCR products on 5% Urea/Polyacrylamide Gel

13/7/6 / GMSF / SF

25 μ g/ml

Epo 1U/ml

37°C

A.A G0 12 μ l LTC-2c

NoDNA	untreated				TdL clasp				dAP		
	1	2	3	4	5	6	7	8	6	60	600 copy
No.1											
No.2											

A.A G1 12 μ l LTC-2c

NoDNA	untreated	TdL clasp				dAP					
		1	2	3	4	5	6	7	8	6	60
No.3											
No.4											

10/91 ()

2 μ l, no colony

c 7 nice colony, 2 cluster on stroma

25 (nice colony)

17 (cluster on stroma a

loose in media)

4 (nice colony)

5 (cluster on stroma

16 (cluster on stroma or floating)

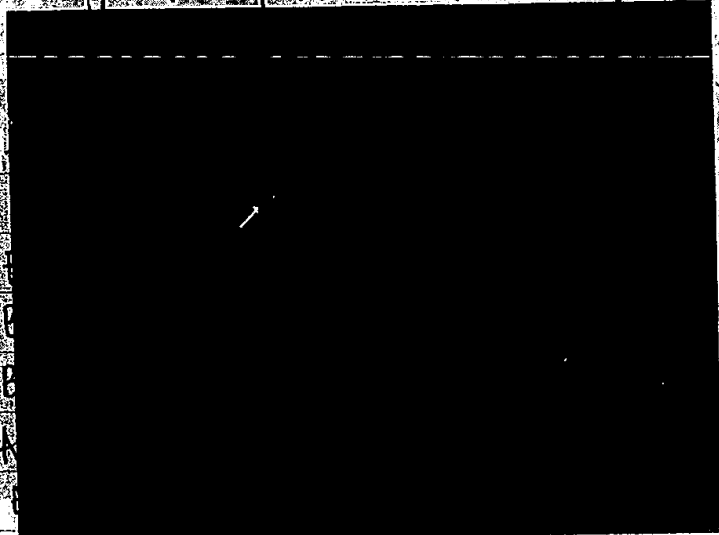
load 1/3

dry 80°C 2 hrs

Hybridized probe on FISH slides were detected.

Td

Harvested:



+IP	-IP	%IP
24	110	18%
25	80	23%
17	117	13%
4	48	8%
4	49	8%
0	16	0%
0	12	0%

Bauer, C

Dropped slides of G. csa (G. ca
G. ca

spred @ -2000.

patients: Billy, V (VB)

Bravo, J (JB)

Ran antisense HIV PCR product on Midi gel 0.8%
Performed Southern blot.

Split 293 and made 4 35mm plates for titrating
my dPAP ccl vector.



Diagen of GFP plasmid.

PPT o/n.

loaded a 100ml culture into one column!

→ overloaded column!

therefore lost a lot of plasmid.

hybridized probe to G₀/G₁ slides.

Resuspended GFP plasmid pellet in TE.

Quantitated

Yield only 60µg/ml.

therefore only a total of 60µg!

but SC still could get 4 plates out of my 60µg.

Went to FISH lab to defect probe.

Experiment #2: FISH

Objective: Determine if aPAP vector actually integrated into genome of transduced CD34+ cells residing in G₀.

Results:

	+MP	-MP	% MP	+IP	-IP	% IP
VB Untd G ₀	-	-	<1%	-	-	<1%
VB (D35) G ₀ -CCL	3	19	14%	30	301	9%
VB (D35) G ₀ -CCL	4	29	12%	22	210	9%
VB (D35) G ₁ -CCL	-	-	N.D.	-	-	N.D.
JB (D23) G ₀ -CCL	5	29	15%	24	232	9%
JB (D23) G ₀ -CCL	5	21	19%	13	82	14%
JB (D23) G ₁ -CCL	4	24	14%	22	178	11%

Deleted probe on First slide.

Results:

	+MP	-MP	%MP	+IP	-IP	%IP
unt dG ₀ (VB)			0%			0%
Td G ₀ cell (VB)	2	20	9%	22	145	13%
Td G ₀ cell (VB)	1	11	8%	25	107	19%
Td G ₀ cell (VB)	1	14	7%	17	105	14%
Td G ₀ cell (VB)	1	15	6%	14	91	13%

Made up MC culture media:

TOTAL Volume = 10mL

MEM 8mL

20% FCS 2mL

IL3 10ng/mL 10 μ L

GMO SF 50ng/mL 2 μ L

Prehybridized Blot.

performed Cindy's MC colony: Hakeford C Crude 2.

→ LTC-10 plate

Trypsin-EDTA: Each 15mL tube, preadded 5mL MEM + 0.5mL FCS. Removed media in wells and placed into tube. Then 0.7mL Trypsin was added and let sit for 1min 15sec. Added 15mL media from 15mL tube to rinse the well. Repeated one more time to clean the well as good as possible. Then added 2mL fresh plain MEM to rinse well.

GOCD34+fish.xls

Pati nt	G0? G1?	Weeks P	st Transducti n	% +ive MP	% +iv IP (<3)	Comments
Billy, V.	G0	4		9% (2/20)	13% (22/145)	VB Td on!
Billy, V.	G0	4		8% (1/11)	19% (25/107)	
Billy, V.	G0	5		14% (3/19)	9% (30/301)	No metaphase spreads
Billy, V.	G0	5		12% (4/29)	9% (22/210)	
Billy, V.	G1	5		N.D.	N.D.	
Billy, V.	G0	6		7% (1/14)	7% (8/105)	
Billy, V.	G0	6		6% (1/15)	9% (9/91)	
Bravo, J.	G0	3		15% (5/29)	9% (24/232)	JB Td on
Bravo, J.	G0	3		19% (5/21)	11% (12/112)	
Bravo, J.	G0	3		18% (4/18)	11% (19/150)	
Bravo, J.	G1	3		14% (4/24)	11% (33/178)	
Bravo, J.	G1	3		16% (3/16)	7% (12/170)	
Bravo, J.	G0	4		10% (3/26)	12% (24/170)	
Bravo, J.	G0	4		14% (4/25)	10% (21/184)	

Pelleted Qiagen prep of ~~total RNA~~

Washed w/ 70% EtOH

Resuspended in a total of 1mL TE
(a 100mL culture!)

Quantitated by spec.

[3] = 0.500 $\mu\text{g}/\mu\text{L}$ \therefore total = 500 μg

Pelleted 293 genomic DNA. in microcentrifuge
@ 15,000 RPM for 40min.

Washed w/ 70% EtOH

Resuspended in 100 μL of TE

Heated sample to allow for better suspension

Quantitated by spec.

[3] = 1 $\mu\text{g}/\mu\text{L}$ \therefore Total = 100 μg
 $260/280 = 1.74$

Also Hybridized probe to FISH slides G.₁/G.₁
(and one Frias, Francisco slide)

Allowed for hybridization o/w @ 37°C in
tissue culture incubator.

Pelleted FISH probe (stored @ -70°C over my
vacation).

Washed w/ 70% EtOH

Dried and Resuspended in a total of
15 μL .

Stored @ -70°C.

Detected product on $en34 + G_0$ slides.

Results:

	+MP	-MP	2-MP	+IP	-IP	2-IP
All Patient JB			<1%			<1%
Untd G ₀						
G ₀ cell ()	3	26	10%	24	170	12%
G ₀ cell ()	4	18	18%	19	158	11%
G ₀ cell ()	4	25	14%	21	184	10%
G ₀ cell ()	3	16	16%	12	170	7%
Fract F	5	20	20%	19	181	9.5%

→ Colcemid blocked FISH brushed culture.

Set up another assay PCR w/ newly extracted 293 DNA.

Genomic @ 100ng/1

		<u>x10</u>
10x Buffer	2.51	201
DMSO @ 10mm	.6251	7.51
RPLP @ 10mm	.6251	7.51
dNTP @ 2.5mm	21	241
Taq	.251	31
MgCl ₂ @ 25mm	21	241
H ₂ O	161	1921

Total rxn = 251

Set Program @ 98° 5'

80° 1'

94° 40"

64° 40"

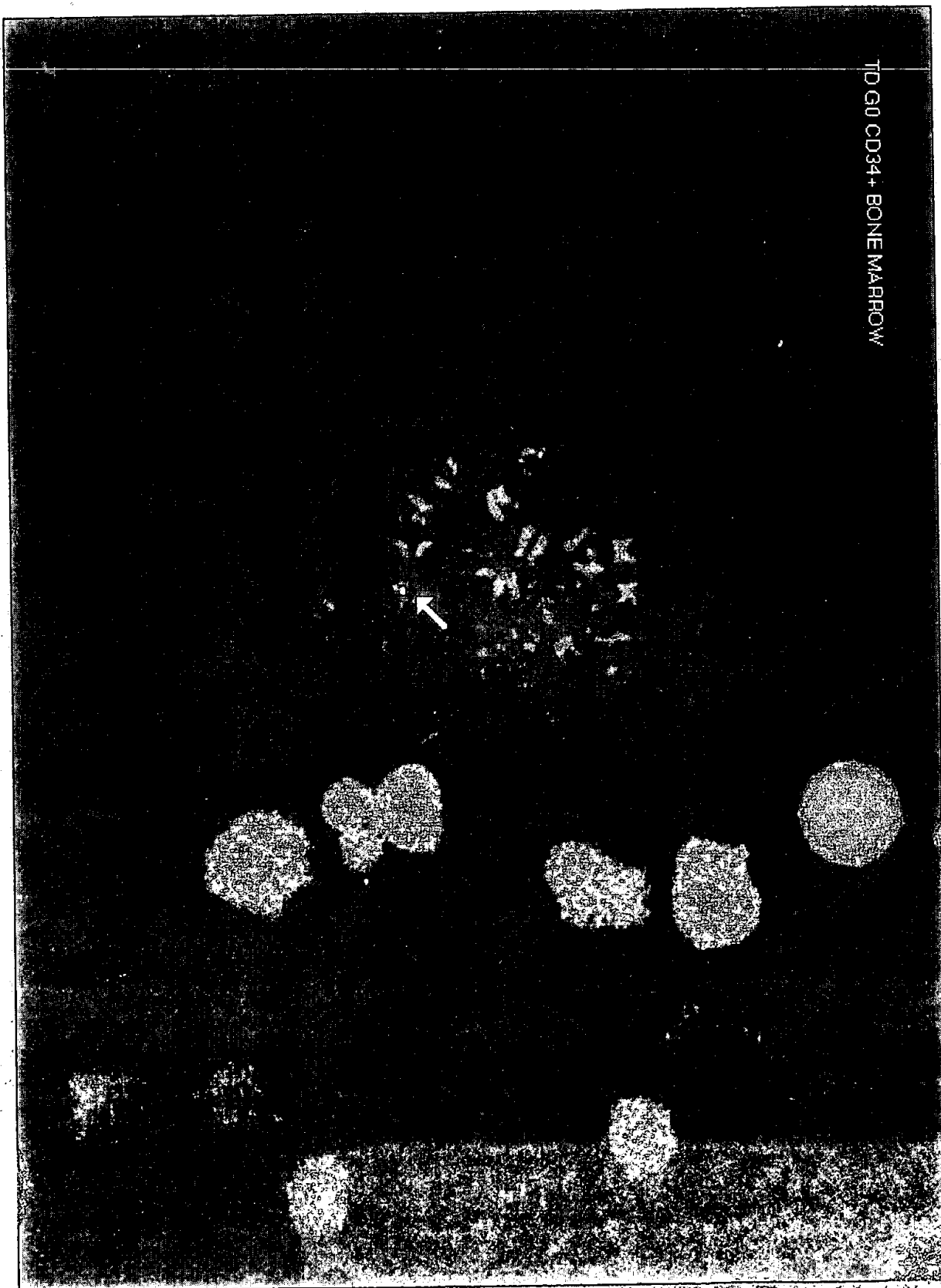
72° 1'

} 30 cycles

70° 5'

4° 2hrs.

TD G0 CD34+ BONE MARROW



UNIT G0 CD34+ BONE MARROW

Bone Marrow today!

Patient:

97-32-31 4
GILLIARD, JEANNETTE
F / W 05/19/1964

Christine

good yield!!!
of CD34+

Total Volume: 20 mL

→ 2 mL for Lu Jiao

→ 18 mL for Isolation of CD34+

Count on Mononuclear cells = 250,000,000 cells.

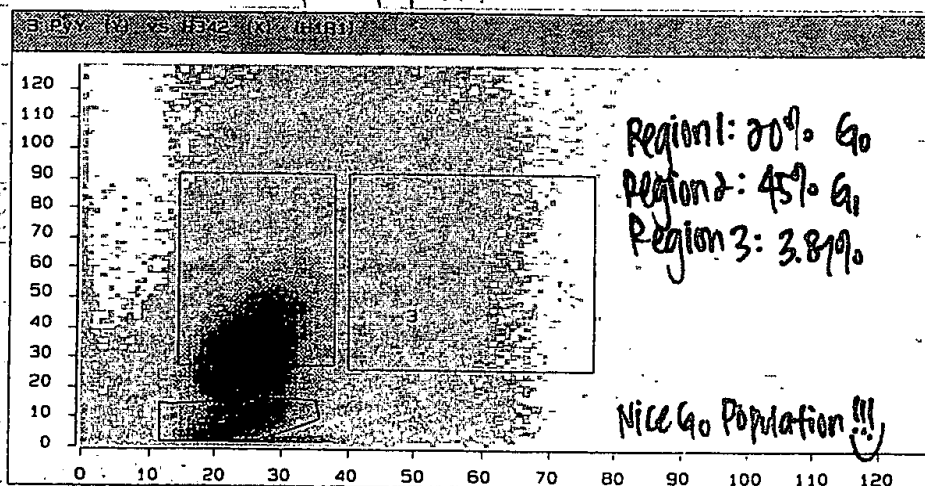
Count of Total CD34+ = 3×10^6 cells.

Stored @ 4° in 50% FCS / 50% IMDM.

→ will select for G₀/G₁ tomorrow!

Made new concentration batch of Hst & Pyronin stain.
Stained as usual.

FACS sort G₀/G₁ populations.



20,000 cells each area

CSA (pooled SCA LL) 30% each well

Sc asked me to calculate % CD34+ from mononuclear cells from donor to donor.

Patient	Volume BM	# Mononuclear	# Total CD34+	% CD34+
Valentino	15ml	1.00E+08	1.60E+06	1.6
Bravo	44ml	4.00E+08	4.30E+06	1.075
Meiser	38ml	2.80E+08	2.50E+06	0.892857
Alexander	30ml	2.00E+08	2.80E+06	1.4
Kanata	50ml	2.70E+08	3.30E+06	1.222222
Thompson	40ml	2.50E+08	3.40E+06	1.36
Gilliard	18ml	2.50E+08	3.00E+06	1.2

∴ approx. 1% CD34+

cells.

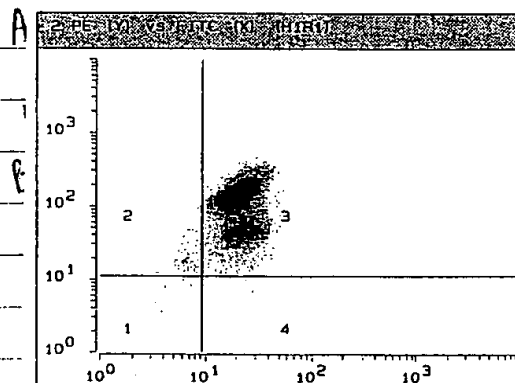
FACS analysis of CD34+CD38- pop. in the G0/G1, CD34+ cells sorted on (Patient: Gilliard, Jeannette).

Washed aliquoted cells in ~100ul of FACS Buffer.

FACS Buffer: PBS + 0.1% Sodium azide
+ 2% FCS

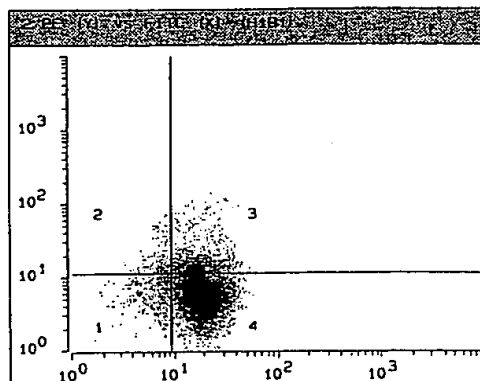
Diluted Ab 4ul in 100ul media. CD34+/CD38- (Becton Dickinson)

in stain.



Status: Panel 1			
EVENTS:	40276 counts		
TIME:	129 secs		
RATE:	312/sec		
SOURCE:	SACAPR17.003		
MODE:	Free Run		
PROTOCOL:	FITCPE.SAC		
LISTMODE:	<none>		
SAMPLE:	G1 CD34/38		
EXPERIMENT:	CD34/38 Staining		

Region	Counts	%
1	161	0.42
2	358	0.93
3	37792	98.30
4	136	0.35



Status: Panel 1			
EVENTS:	40521 counts		
TIME:	157 secs		
RATE:	253/sec		
SOURCE:	MOFLO		
MODE:	Listmode 50000		
PROTOCOL:	FITCPE.SAC		
LISTMODE:	1\SACAPR17.001		
SAMPLE:	G0 CD34/38		
EXPERIMENT:	CD34/38 Staining		

Region	Counts	%
1	1757	4.66
2	1062	2.82
3	7386	19.60
4	27479	72.92

20% G0
45% G1
∴ 3.81%

○

○

○

Majority of G0 pop. is CD38-.

Majority of G0 pop. is CD38-.

now.

Washed & fac cp34+ cells Patient: Alexander, Tracy
→ Replated & replaced @ 37°C

Fish Brooked Total cp34+ (1 wk well)

Colomud Brooked cp34+ CM (Go/en)

Dropped CM slides (2wk) → Go/G,
Could not Harvest Encapsidation Today!!
→ CPE not up to 50% yet.

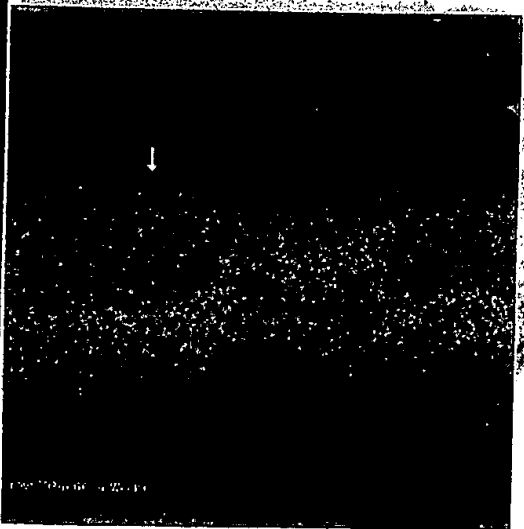
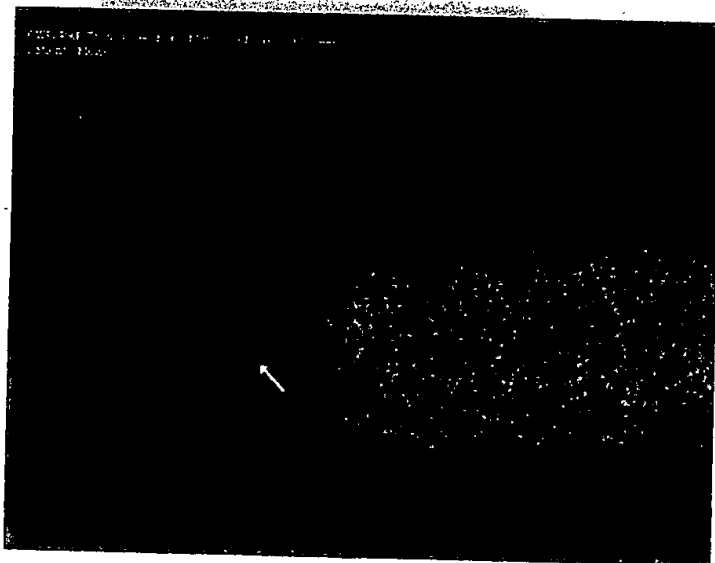
Harvested Encapsidation.

2 tubes. (5 plates/tube)

Proceeded w/ Protocol as described by challenge/vary.
After adding FCS, stored @ -80°C

Dropped TM slides (1wk) → Total cp34+

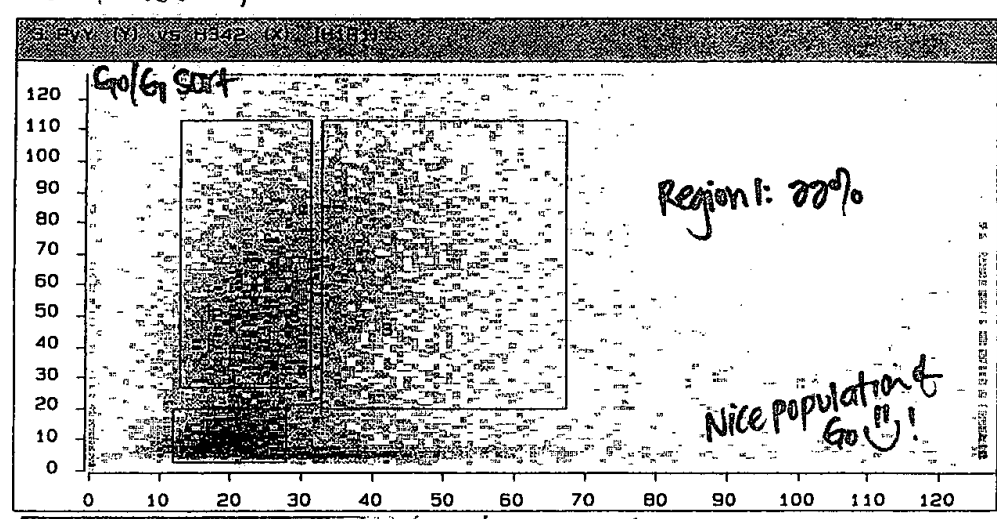
Hybridized dPAP probe to 2 week/4 week Go CP34+
Patient: Meier, Cynthia



of CD34+ cell = 2.5 million
gave 500,000 to Priscilla.

Shred @ 40 in 50% isozyme / 50% FCS O/N.

Stained CD34+ w/ Hrechat & Pyronin.
(Before staining aliquoted 80,000 out total CD34+ cells)



May 80,000 G0 100,000 G1
Me 70,000 G0 70,000 G1

Set up my wells as follows:

plate 1:

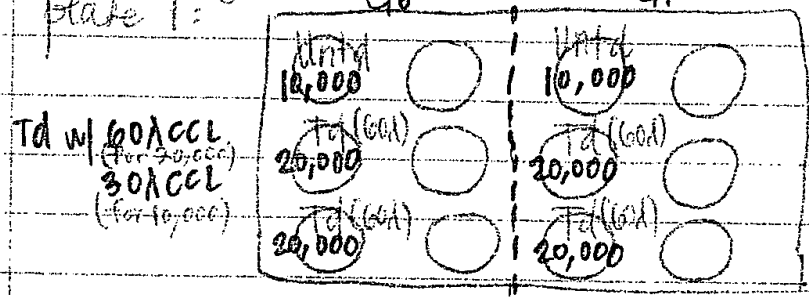
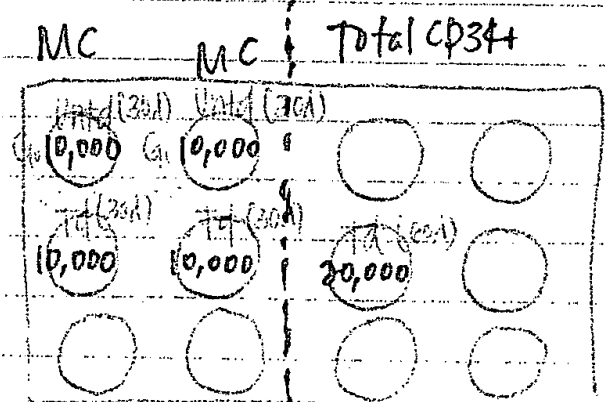


plate 2:



FACS sort G_0/G_1 populations after staining
w/ Hoechst/Pyrromu

After sort,

$$G_0 = 144,575$$

$$G_1 = 163,326$$

Divided cells among Cindy and I.

Cindy 80,000 G_0 100,000 G_1

Me 70,000 G_0 70,000 G_1

Set up my wells as follows:

plate 1:

G_0	G_1
Untd 10,000	Untd 10,000
Td (60A) 20,000	Td (60A) 20,000
Td (60A) 20,000	Td (60A) 20,000

Td w/ 60A CCL
(for 20,000)
30A CCL
(for 10,000)

plate 2:

MC	MC	Total CP34
Untd (30A) 10,000	Untd (30A) 10,000	
Td (30A) 10,000	Td (30A) 10,000	

(5d) ..
nd shield.

Frozen/thawed 2x → Next 2x more.

Good card box @ 30c ea.

Adjusted pH of the stock to 8-8.5 using sterile 1M Tris pH 9.5.

Added Mn^{2+} \rightarrow final $\text{mm} = 11$

Added 4.5% Bionase 00'

4.51 Pressure another 60'

Added 1.1 mL 10X trypsin \rightarrow 1X

Added 1.1 mL DUC 10% \rightarrow 1%

Vortexed.

Incubated @ 37°C for 30'

Gonicated.

Added $\sim 6.9 \text{ g CsCl}$

Adjusted PI to 1.4 (density).

Loaded onto centrifuge w/ CV, lai (2), SO, SC pooled in mine!!!

Will harvest

301 (20,000 cells)

Week 8

Week 6

Week 4

6005-102501
FISH HARVEST

Reproduced on 7.8.98

CD34+CD38- cells...

Dominor: Mercado, O

vector

checked Centrifuge OK ✓

Bone Marrow Today!

Cindy isolated CP34+ $\sim 8 \times 10^6$ cells!

Patient : Mercado, Orfelia 98-26-16-5

F/W 03/22/1910

BFRB
ul
480
480
480

CP34/CP38-Sort.

Resuspended FAES Buffer:

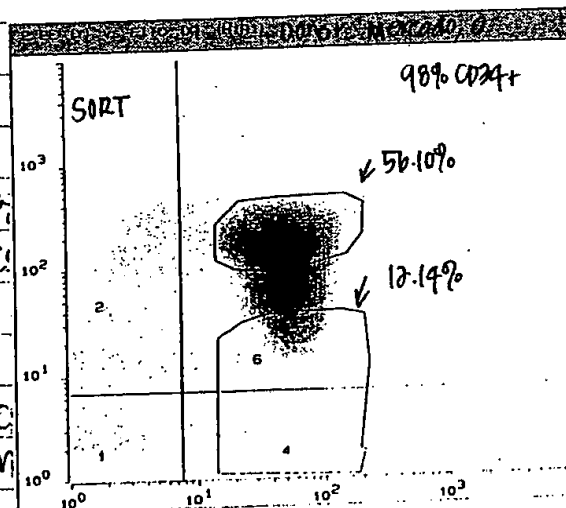
diluted 50% Ab \rightarrow 75% FAC!

Mixed into same tube.

Incubated on ice for 45'

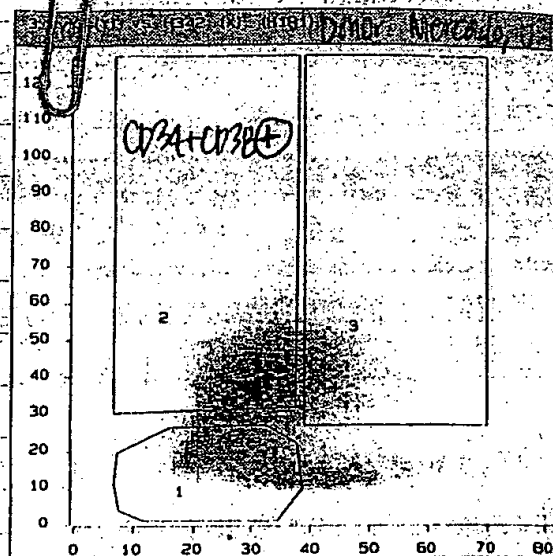
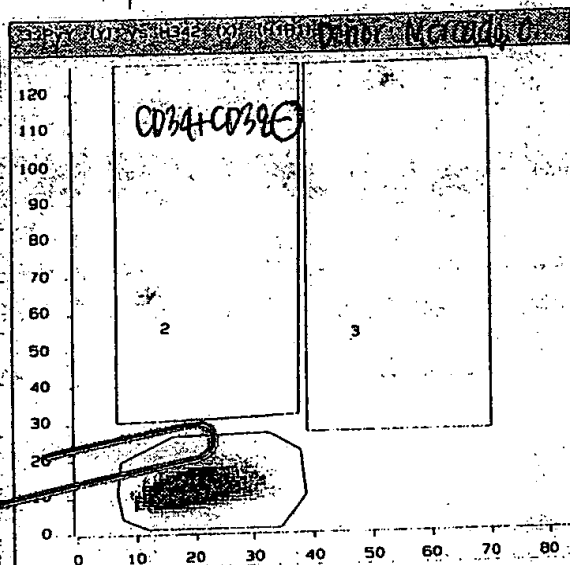
washed w/ 2.5 ml FAE

TRW CN 5/98 CSCI v



292

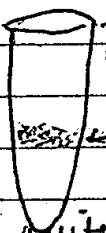
G0/G1 analysis of CD34+CD38- population.



→ Definitely 2 different cell populations. 98% of all CD34+CD38- cells are in G0!!!

Gradient... cu

Besmae
worked
too well???



totally clear!

disposed faint band

Density is quite high!!!

VECTOR HARVESTED	CWRAPAP 7/9/98		
FRACTION	RI	DENSITY	COMMENT
1	1.3576	1.2463	TOP
2	1.3586	1.2571	
3	1.3615	1.2886	
4	1.363	1.3049	
5	1.365	1.3266	
6	1.3674	1.3527	
7	1.377	1.4570	Vector Band
8	1.3771	1.4580	Vector Band
9	1.3775	1.4624	Vector Band
10	1.3725	1.4081	
11	1.386	1.5547	
12	1.3906	1.6047	

Bone Marrow Today!

Patient:

98-29-18 5
LEAGS, RICARDO
H / W 12/20/1976

Christine